THE BREAKING-UP OF HIBERNATION IN THE CODLING MOTH LARVA

BY

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Introduction.

Hibernation has been frequently studied with a view to determining what factor or factors were responsible for producing the dormant condition. In the present paper the problem has been attacked with a view to determining what brings about the break-up of the dormancy in spring. The experimental data thus gained indicate that hibernation in the codling moth is more than a condition of passive resistance to cold weather and other unfavorable conditions. During the cold winter months changes are going on in the tissues of the animal which are necessary for the further functioning of the organism and hibernation is a necessary stage in the life history of the insect.

The experimental data given below, when correlated with the work of others, justify the theory that the most important factor in the breaking up of hibernation in cold-blooded animals is the reabsorption of water by the tissues. This reabsorption speeds up the enzyme activity of the tissues and the animal becomes active again. A return of warm weather in the spring is of course also important, but this factor is always experienced by a hibernating animal as the season advances. Whether the animal can absorb the requisite amount of water depends upon the local situation in which it hibernated, and without this addition of water, the enzymatic processes necessary for renewed activity cannot take place.

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MATERIALS AND METHODS.

Codling moth larvæ of the second generation were collected in the vicinity of Urbana, Illinois, during the summer of 1923. Six-inch tar paper bands were tacked around the tree trunks for collecting the larvæ, and these were examined from time to time during the fall. The larvæ were removed from their cocoons with forceps and dropped into small cardboard boxes, which had been previously prepared by placing in the bottom of each a layer of small glass tubes. The tubes were approximately ¼ inch in diameter and one inch long, and were stood on end and packed tightly into the bottom of the box. The larvæ showed a strong tendency to crawl into the tubes and spin cocoons there, and these tubes of cocoons were later removed from the boxes for use in the experiments.

A field note-book was kept during the season, containing data as to when bands were placed on the trees, when larvæ were collected, number of rainy days, cold nights, etc.

Experiments were performed in the vivarium building, of the University of Illinois, using three temperature chambers, one running at 0°C., one at 10°C., and one at 30°C. Recording thermographs were operated constantly in each chamber and the records showed no variations which might have affected the work. It was found that storage at 0°C. caused the lowest mortality among the larvæ; altho as shown later in the paper, exposure to 10°C. for a time was most conducive to pupation and later progress.

Records of pupations, emergences, etc., were kept for individual larvæ by labeling the tubes either with a glass pencil or a small sticker.

DISCUSSION.

It has been shown by numerous workers that the beginning of hibernation in cold-blooded animals is often marked by a reduction of the water content of the organism, and it is equally true that the breaking-up of hibernation in these forms is usually marked by a taking in of water. This difference in water content is perhaps the most universally recognized difference between the hibernating and non-hibernating animals among the insects and related groups, and may well serve as the starting point for a theory of hibernation.

Tower (1906) described the preparation for hibernation in the potato beetle and showed that the animal eliminated 27% of its weight as watery material during a period of three to ten days and then burrowed in the ground and hibernated. In the spring this water was regained and the protoplasm became more liquid again. Breitenbecker (1918) showed that dessication of potato beetles caused them to pass into a state of "induced" hibernation. According to Bodine, (1923) in grasshoppers which hibernate, such a condition was marked by a lowering of the water content to a minimum, and the breaking up of hibernation was marked by a return to the normal condition in this respect.

Several workers have shown that the addition of water tends to break up dormancy. Thus Breitenbecker found that the addition of water to the soil would cause hibernating potato beetles to emerge, if the temperature was above 14°C. If the soil was never wet the beetles died instead of emerging. Along this same line Weese has shown that young spiders emerge from the winter cocoons only under conditions of high humidity of the surrounding air.

In investigating the winter brood of the codling moth larvæ in the vicinity of Urbana, Illinois, I found that soaking the larvæ at intervals (2 hours per week), caused them to pupate more readily than if they were not soaked, Pupation in this case marks the end of the dormant period and the return to functional activity. The main points of this phase of the work are shown in Table 1 from which the following conclusions may be drawn:

1. Soakings increase the per cent of pupation, whether the water is applied at low temperature (10°C.) or at higher temperature (30° C.) or both.
2. Soakings at 10°C. shortens the length of time to pupation.
3. Soakings at 30°C. shortens the length of time from pupation to emergence.

From these conclusions it seems evident that some process goes on at 10°C., is affected by soakings and is intimately concerned with *pupation.* Also some similar process goes on at 30°C., is affected by soakings and is intimately concerned with emergence or in other words with the later stages of metamorphosis. Such soakings would be normally experienced

by the animals during the spring rains, and probably are effective by reason of their action upon the enzymes of the body tissues and fluids.

That water-content has a marked effect upon pupation processes is further shown by Table 2 where the data on all larvæ experimented upon have been tabulated with regard to this one factor, disregarding other points in the histories of the various groups. Reference to column 3 shows that the greatest percent of pupation occurs among animals soaked frequently at the rate of two hours per week.

TABLE I.

Showing effects of soaking hibernating codlin moth larvæ at different temperatures. Storage temperature 10°C. Pupation temperature, 30°C.

1 2-hr. soakings during storage	2 Number 2-hr. soakings at 30°C.	3 % Pupated	4 Days to pupation at 30°C.	5 Number pupae on which column 4 is based	6 Days to emergence at 30°C.	7 Number emergences on which column 6 is based	8 Days to death of larvae at 30°C.	9 Number of dead larvae on which column 8 is based
5	5	36.0	23.0	8	8.0	2	30.6	14
0	5	33.0	26.0	16	7.0	9	14.8	10
5	0	32.7	23.2	17	10.8	14	25.8	12
0	0	6.6	29.0	1	9.0	1	21.5	14

The effect of water-content upon enzymatic action is well known. According to Fischer, (1907), "In a concentrated solution the point at which the reaction (of digestive enzymes) comes to a standstill is reached sooner than in a more dilute one. In fact most fermentation mixtures which have come to a standstill will go further only if water is added."

Bayliss in his monograph on enzyme action emphasizes the importance of water in such processes. According to this author, "The greater number of enzymes have a hydrolytic action, and their activities are as a rule manifested in the presence of water." Again "an enzyme action comes to an end or equilibrium point owing to the accumulation of the products of the reaction, so that by *dilution* or removal of the products the reaction may be caused to go on further."

The breaking-up of dormancy in the codling moth larva is marked by the metamorphosis of the insect. Such metamorphosis consists morphologically of two processes, namely a process of autolysis of larval tissues, followed by a process of growth and development of adult organs. From a chemical standpoint, then, pupation of the codling moth larva in the spring is initiated by a process of autolysis. This autolysis is probably due to the action of enzymes, an hypothesis to be tested more carefully by later work. A consideration of some data available at this time lends indirect support to this view.

TABLE II.

Summary of results of all codling moth experiments grouped together to show effects of moisture alone, pupation experiments at 30°C.

1 Storage Conditions	2 No. larvae	3 % pupated	4 Days to pupation	5 Number pupae col. 4	6 Days to emergence	7 Number emergences col. 6	8 Days to death, larvae	9 Number of larvae on col. 8
Never soaked	76	24.6	19.9	15	10.6	8	30	45
Soaked once for 15 hrs..	1148	8.5	22.4	48	11.3	31	32.9	274
Soaked several times at rate of 2 hr. per week.	350	50.8	20.7	123	9.5	90	26.2	171

Recent writers have emphasized chiefly the action of enzymes or of acids in bringing about the breaking down of such tissues. Thus Weinland (1909) found that the formation and destruction of fat in the larva of Calliphora was a process governed by an equilibrium condition under the action of enzymes. Simon (1904), Cole (1919) and others mention enzymes as being responsible for autolytic processes in tissues. On the other hand Bishop (1923), has shown that the accumulation of acid in the body of the honeybee larva was probably the primary cause of autolytic processes there, and that enzymes were not important until later.

Whether autolysis in an insect larva is brought about by acids or by an enzyme, the addition of water would be expected

to speed up the action and increase it. Thus Bishop in his discussion of the metamorphosis of the honey-bee larva, where autolysis is due largely to acid, says "when the tissues are diluted with pure water, the buffering action of the mixture is lowered, and a slight excess of acid in one tissue or another would accelerate its rate of autolysis."

We have already noted that dilution has a marked effect upon enzyme action. It is well to remember in this connection that the codling moth larva and the bee larva pupate under quite different conditions of temperature, etc.

TABLE III.

Showing rate of pupation in samples of codling moth larvæ from the same collection, placed under pupation conditions on successive dates during the winter. The first column shows storage temperature and days in storage. At the close of storage the larvæ were placed in 30°C. for pupation. The number of larvæ used was 10 except in one case where 18 were used. The stock of the second item marked with an asterisk, 29.3% pupated at 22°C., indicating that the higher temperature (30°C.) is not essential. However, all this group had undergone preparation for pupation at the lower temperatures experienced in the field before collection.

T; da.	% pupation	Da. to pupation	Da. pupal Life
22°; 9	30	22	12
*22°; 58	20	......	......
22°; 100	0	......	......
10°; 9	50	23	11
10°; 58	30	21	9
10°; 100	27.7	14	......
0°; 9	40.0	21	9
0°; 58	20.0	19	11
0°; 100	25.0	14	......

That the autolytic process in the hibernating codling moth larva is probably due to an enzyme rather than to accumulation of acid seems probable from some of my experimental data, altho as yet no real chemical tests have been made. Experiments show that larvæ kept at low temperatures for considerable periods do not pupate but do undergo some sort of preparation for pupation, so that when placed at 30°C. later, they pupate more rapidly as a result of their treatment at low temperature. Thus, as shown in Table 3, larvæ collected and stored at 10°C. or 0°C. showed increased speed of pupation when placed at 30°C, later in the season. Speed of pupation is indicated in column 3 of Table 3. Such exposure to low tem-

peratures appears to be the usual pre-requisite to the breaking up of hibernation in the codling moth larva, and evidently allows some sort of preparation process to go on.

It will be noted from Table 3, column 2, that larvæ stored at 22°C over a period of time lost their power to pupate. This suggests that the necessary preparation cannot go on at this temperature. Some data on this point are available. Forty larvæ collected August 11 were never exposed to temperatures below 13°C. either in the orchard or the laboratory. Later they were placed at 30°C. but showed no pupation.

195 larvæ which had never been exposed to temperatures below 22°C. in the laboratory showed only 3½% pupation

TABLE IV.

Showing effects of exposure of codling moth larvæ to low temperatures for varying periods of time. Pupation temperature, 30°C.

Number Larvae	Low Temperature	at Low Temperature	Percent of Pupation
106	0°C.	7–14 days	6.6
74	0°C.	30 days	28.0
220	10°C.	7–14 days	25.4
72	10°C.	30 days	8.3

when placed at 30°C., and this slight percent may be explainable by a slight exposure to low temperature in the orchard before collection as shown by field records.

Evidently then in the hibernating larvæ, some process which is a prerequisite to pupation goes on only at low temperatures. Table 4 gives some data regarding the effect of low temperatures on this process. It shows that the process goes on rather slowly at 0°C. and considerably faster at 10°C. indicating a maximum activity for the process at about 10°C. Furthermore exposure to 10°C. if continued long enough may prove detrimental. Exposure for as long as 30 days may undo some of the work accomplished during the first two weeks.

The fact that preparation for pupation does go on slowly at as low a temperature as 0°C. is of considerable interest,

and indicates that the metabolic activity of the animal is not readily brought to a standstill by winter conditions. The effects of exposure to different temperatures is further shown in Table 5, where the data regarding all larvæ used in the experiments have been tabulated with regard to this one factor. As shown in Column 2 of this table, percent of pupation was highest in larvæ stored at 10°C., lowest among those at 22°C.

It seems likely that if the process of autolysis of larval tissues was due to acidity it would be speeded up by higher temperatures, whereas the data given above indicate that in the winter brood of codling moth larvæ the process does not

TABLE V.

Summary of results of all codling moth experiments grouped together with regard to storage temperature alone. Pupation and larval death temperatures, 30°C.

1 Storage	2 % pupation	3 Day to pupation	4 No. pupae col. 3	5 Pupal Life, Days	6 No. col. 5	7 Days to death of larvae	8 Dead larvae col. 3	9 Total larvae	10 Aver-% of mortality, storage
22°C.	8.1	21.9	40	8.4	28	35.6	358	1141	56
10°C.	52.6	21.1	146	9.5	108	26.9	233	452	38
0°C.	31.2	17.8	10	9.6	6	28.1	16	32	0

go on at all at temperatures above 22°C. An enzyme on the other hand might well have a maximum activity at a temperature as low as 10°C. especially in a cold-blooded aimal like an insect. Accordingly we may suppose that in the hibernating codling moth larva autolysis of larval tissues is brought about by an enzyme which works best at temperatures around 10°C. Such a hypothesis can be tested by later work. On this basis the exposure of the animal to low temperatures during the hibernating period is not a case of mere resistance to unfavorable conditions but in part at least is a period necessary for certain vital functions to go on.

If the above hypothesis is correct, i. e., that changes in larval tissues are the result of an enzymatic process, affected by soakings and having a temperature maximum of about 10°C., we should expect that a combination of these two factors

in the environment would give the maximum effects. Table 6 shows the available data on this point. The table is incomplete as regards some groups, but the following conclusions may safely be drawn from it.

1.—Percent of pupation at 30°C. is—
Greatest after exposure to 10°C., soaked frequently
Next(lower) " " " 10°C., " once.
Next(lower) " " " 22°C., " "
Least " " " 22°C., dry.

2.—Number of days to pupation at 30°C. is—
Shortest after exposure to 10°C., soaked frequently.
Next " " " 22°C., " once.
Longest " " " 10°C., " "

3.—Number of days from pupation to emergence at 30°C. is—
Shortest after exposure to 22°C., soaked once.
Next " " " 10°C., " "
Longest " " " 10°C., " frequently.

Table VI.

Showing effects of storage temperature and moisture upon hibernating Codling moth larvæ. All pupation experiments at 30°C.

1 Storage Conditions (Temperature and moisture)	2 No. larvae	3 No. pupa-ted	4 % pupa-ted	5 Days to pupa-tion	6 No. pupae col. 5	7 Days to emer-gence	8 No. emer-gen-ces, col. 7	9 Av. da. to death larvae	10 No. larvae col. 9
10°C. Dry..........	38	12	34.2	19.3	12	10.4	7	30.5	19
10°C. Soaked once for 15 hrs.....	58	8	13.8	25.0	8	9.0	7	24.1	38
10°C. Soaked several times 2 hrs. per week.	350	178	50.8	20.7	123	9.5	90	26.2	171
22°C. Dry..........	32	3	10.3	?	0	?	0	21.9	26
22°C. Soaked once for 15 hr......	1,090	90	12.1	21.9	40	8.6	24	34.3	236

We see, then, that the greatest percent of pupation and fastest speed in the process goes on at 10°C. soaked frequently, and this is in agreement with our hypothesis. In other words frequent small soakings at 10°C. furnish optimum conditions for the enzymatic processes which precede pupation. The cold, soaking rains of winter and spring would furnish ideal conditions for preparing the animal for renewed activity as soon as the weather warms up a little.

The summer brood of codling moth larvæ do, however, pupate at higher temperatures, and such an enzyme would not be able to work under such conditions. This is obviously a different case, since continued exposure to summer temperatures has no effect upon the winter brood, as far as pupation is concerned. We may perhaps explain the autolysis of larval tissues in this case on the basis of acidity. We have already noted that Bishop, (1923), found that in the bee larva autolysis is due largely to the increased acidity of the tissues due to the activity of spinning, assimilation of fats, etc. The case of the bee larva is similar to that of the summer brood of the codling moth larvæ in that the metamorphosis is rather rapid and goes on at a rather high temperature. The case of the summer brood has not yet been exhaustively studied from this point of view.

In the case of the codling moth and doubtless in others not yet studied in this way, it is evident that addition of water to the tissues tends to break up the dormant period and renew life processes. The renewed activity of the animal may be shown in various ways in different species—by metamorphosis in the case of the codling moth; by growth and activity in the case of young grasshoppers or in certain spiders; or in the case of animals which hibernate as adults, by simply renewed activity, especially as regards the sexual organs. Perhaps further work will show that in these latter cases addition of water has a marked effect upon the enzymatic processes in the organs of generation which are usually most active in the spring.

SUMMARY.

1.—The break-up of hibernation in the codling moth larva is hastened and aided by the addition of water to the tissues, such as normally takes place during rains. This water, by diluting the fluids, probably speeds up the enzyme action in the animal's body, and the result is a renewal of metabolic processes, pupation, and general activity. The frequency of soaking is of much importance.

2.—During the exposure to rather low temperatures in winter and spring the tissues of the winter brood of codling moth larvæ undergo a preparation for pupation which marks the renewal of activity in spring.

3.—The preparation process does not go on at temperatures as high as 22°C. 10°C. is more favorable for it than 0°C. It may be explained by assuming the presence of an autolytic enzyme in the tissues, which works best at a temperature near 10°C.

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